

## Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- |                                     |                                     |  |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of all covariates tested   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated   |

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

Data collection

Data collection:

- For flow cytometric data collection, the BD FACSDiva Software (v9.1) software was used
- For confocal microscopy data collection, the Leica LAS X Version 5.0.2 was used
- For brightfield microscopy data collection, the Zen 2 (blue) edition software was used
- For qRT-PCR data collection: the QuantStudio Analysis Software (ver 1.7.1) was used
- For plate reader assays, Spark Control Software InfiniteM1000 Pro

## Data analysis

• For RNA sequence analysis: The Partek flow [1] and Ingenuity Pathway Analysis (IPA) [2] were used for bioinformatics methods and data analysis. Reads per gene were quantified using STAR - 2.7.2a [3] and mm10 (Ensembl GRCm38.97).

[1] Partek® Flow® software, version 7.0 Copyright ©; 2019 Partek Inc., St. Louis, MO, USA.

[2] IPA: Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014 Feb 15;30(4):523-30.

[3] STAR: ultrafast universal RNA-seq aligner, A Dobin, CA Davis, F Schlesinger, J Drenkow, C Zaleski, S Jha, P Batut, Bioinformatics 29 (1), 15-21 Fast gapped-read alignment with Bowtie 2. Langmead B, Salzberg SL. Nat Methods. (2012)

- For flow cytometric analysis, FlowJo (version 10.7.1) was used
- For microscopy analysis, Fiji (version 2.1.0/1.53c) was used
- For statistical analysis, GraphPad Prism (version 9.1.0) was used
- For surface plasmon resonance, Pioneer QDAT software (Version 3.41) was used

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The raw data files of RNA sequencing data have been deposited and are openly available at the Gene Expression Omnibus GEO data repository under accession code (GSE149776, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE149776>). The MetazSecKB database is available at <http://proteomics.yzu.edu/secretores/animal/>. All other data supporting the findings of this study are openly available within the Source Data File included in this manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

### Sample size

For all animal survival studies a sample size of n = 12 mice was used to achieve a 95% power to detect a mean difference of 1 standard deviation with a significance level of 0.05 using a Log-rank (Mantel-Cox) test. Competitive repopulation assays were performed with a sample size of n = 10 mice and hematopoietic recovery studies were performed with n = 8 - 9 to achieve a 95% power to detect a mean difference of 1 standard deviation with a significance level of 0.05 using a two-tailed student t-test. Sample sizes were also contributed to by independent replicate experiments.

### Data exclusions

No data were excluded from the analysis.

### Replication

All attempts at replication were successful. All experiments were repeated in multiple independent experiments. Within the figure legends, the number of replicates is defined.

### Randomization

All mice used in this study were weight, sex and age matched prior to their allocation into treatment groups.

### Blinding

No blinding was performed because the data acquired were obtained by using objective quantitative methods and researchers did not have expectations regarding the outcome.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

## Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

## Antibodies used

## Antibodies

Goat anti-Nrp1 (1:200)

Validation criteria: [https://www.rndsystems.com/products/mouse-rat-neuropilin-1-antibody\\_af566](https://www.rndsystems.com/products/mouse-rat-neuropilin-1-antibody_af566) R&D Systems Cat. AF566; RRID:AB\_355445

Anti-mouse VE-Cadherin Alexa Fluor® 647 (1:200)

Validation criteria: <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-cd144-ve-cadherin-antibody-6569> Biolegend Cat. 138006 RRID:AB\_10569114

Anti-Leptin receptor PE (1:200)

Validation criteria: <https://www.biossusa.com/products/bs-0961r-pe> Bioss Antibodies Cat. bs-0961R-PE RRID:AB\_11051477

Rat Anti-Mouse B220 (CD45R) APC-Cy7 (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-rat-anti-mouse-cd45r.552094> BD Biosciences Cat.552094 RRID:AB\_394335

Rat Anti-Mouse CD45 V450 (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/v450-rat-anti-mouse-cd45.560501> BD Biosciences Cat.560501; RRID:AB\_1645275

Rabbit Anti-P53 (2µl/106 cells)

Validation criteria: [https://www.cellsignal.com/products/primary-antibodies/p53-7f5-rabbit-mab/2527?site-search-type=Products&N=4294956287&Ntt=2527s&fromPage=plp&\\_requestid=2757103](https://www.cellsignal.com/products/primary-antibodies/p53-7f5-rabbit-mab/2527?site-search-type=Products&N=4294956287&Ntt=2527s&fromPage=plp&_requestid=2757103) Cell Signaling Cat: 25275 RRID:AB\_10695803

Mouse Anti-Phospho P53 (S15) (2µl/106 cells)

Validation criteria: [https://www.cellsignal.com/products/primary-antibodies/phospho-p53-ser15-16g8-mouse-mab/9286?\\_id=1635037496378&Ntt=9286S&tahead=true](https://www.cellsignal.com/products/primary-antibodies/phospho-p53-ser15-16g8-mouse-mab/9286?_id=1635037496378&Ntt=9286S&tahead=true) Cell Signaling Cat: 9286S RRID:AB\_331741

Mouse Anti-CD45.2 FITC Mouse anti-Mouse (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fits-mouse-anti-mouse-cd45-2.553772> BD Biosciences Cat.553772 RRID:AB\_395041

Anti-Mouse CD45.1 Brilliant Violet 605 (2µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd45-1-antibody-7850> Biolegend Cat.110738 AB\_10863973

Rat Anti-Mouse Gr-1 (Ly-6G and Ly-6C) PE (2µl/106 cells)

Validation criteria: [https://www.bdbiosciences.com/content/dam/bdb/products/global/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/553128\\_base/pdf/553128.pdf](https://www.bdbiosciences.com/content/dam/bdb/products/global/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/553128_base/pdf/553128.pdf) BD Biosciences Cat.553128 RRID:AB\_394644

Rat Anti-Mouse Mac-1 (CD11b) PE (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-cd11b.557397>

BD Biosciences Cat.557397

RRID:AB\_396680

Rat Anti-Mouse CD3 V450 (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/v450-rat-anti-mouse-cd3-molecular-complex.561389>

BD Biosciences Cat.561389

RRID:AB\_10679120

Anti-Mouse CD41 Alexa Fluor 488 (2µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-cd41-antibody-6907>

Biolegend Cat.133908

RRID:AB\_10645332

Rat anti-Mouse CD150 Alexa Fluor 647 (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-647-rat-anti-mouse-cd150.562647>

BD Biosciences Cat.562647

RRID:AB\_2737701

Anti-Mouse CD34 Antibody PE (5µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/pe-anti-mouse-cd34-antibody-3072>

Biolegend 119308

Anti-Mouse CD34 Alexa Fluor® 647 (5µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-cd34-antibody-3773>

Biolegend Cat: 119314

Rat anti-Mouse CD117 APC-H7 (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-h7-rat-anti-mouse-cd117.560185>

BD Biosciences Cat: 560185

Rat anti-Mouse c-kit (CD117) PE (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-mouse-cd117.553355>

BD Biosciences Cat.553355

Anti-mouse Ly-6A/E (Sca-1) Brilliant Violet 605™ (2µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-ly-6a-e-sca-1-antibody-8664>

Biolegend Cat: 108133

Rat anti-Mouse Sca-1 (Ly-6A/E) APC-Cy7 (2µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-rat-anti-mouse-ly-6a-e.560654>

BD Biosciences Cat.560654

RRID:AB\_1727552

Lineage Antibody Cocktail V450 Mouse (20 µl/106 cells)

Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/panels-multicolor-cocktails-ruo/v450-mouse-lineage-antibody-cocktail-with-isotype-control.561301>

BD Biosciences Cat.561301

RRID:AB\_10611731

Anti-Mouse CD31-Alexa-647 (5µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-cd31-antibody-3094>

Biolegend Cat. 102516

RRID:AB\_2161029

Anti-Mouse CD31-Alexa-488 (2µl/106 cells)

Validation criteria: <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-cd31-antibody-3091>

Biolegend Cat. 102414

Anti-CD31 Alexa-488 (5µl/106 cells)

Validation criteria: [https://www.rndsystems.com/products/mouse-rat-cd31-pecam-1-alexa-fluor-488-conjugated-antibody\\_fab3628g](https://www.rndsystems.com/products/mouse-rat-cd31-pecam-1-alexa-fluor-488-conjugated-antibody_fab3628g)

R&D Systems Cat. FAB3628G  
RRID:AB\_10972784

Anti-Endomucin AF750 (2µl/106 cells)  
Validation criteria: <https://www.biossusa.com/products/bs-5884r-a750>  
Bioss Antibodies Cat. bs-5884R-a750  
RRID:AB\_11090525

Annexin V-FITC (2µl/106 cells)  
Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/panels-multicolor-cocktails-ruo/fitc-annexin-v-apoptosis-detection-kit-i.556547>  
BD Biosciences Cat: 556547

Anti-Mouse LGR5 Alexa-488 (2µl/106 cells)  
Validation criteria: [https://www.rndsystems.com/products/mouse-lgr5-gpr49-alexa-fluor-488-conjugated-antibody-803420\\_fab8240g](https://www.rndsystems.com/products/mouse-lgr5-gpr49-alexa-fluor-488-conjugated-antibody-803420_fab8240g)  
R&D Systems Cat: FAB8240G-100UG

Anti-LGR5 (5µl/106 cells)  
Validation criteria: <https://www.abcam.com/lgr5-antibody-oti2a2-ab273092.html>  
Abcam Cat: ab273092

FITC Mouse Anti-Ki-67 Set (2µl/106 cells)  
Validation criteria: <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/panels-multicolor-cocktails-ruo/fitc-mouse-anti-ki-67-set.556026>  
BD Biosciences Cat: 556026  
RRID:AB\_396302

Anti-Phospho-Cdk5 (Tyr15) (2µl/106 cells)  
Validation criteria: <https://www.origene.cn/catalog/antibodies/primary-antibodies/ta325347/cdk5-rabbit-polyclonal-antibody>  
Origene Cat: TA325347

Recombinant Anti-Semaphorin 3A antibody [EPR19367] (5µl/106 cells)  
Validation criteria: <https://www.abcam.com/semaphorin-3a-antibody-epr19367-ab199475.html>  
Abcam Cat: ab199475

Recombinant Anti-Neuropilin 1 antibody [EPR3113] (5µl/106 cells)  
Validation criteria: <https://www.abcam.com/neuropilin-1-antibody-epr3113-ab81321.html>  
Abcam Cat: ab81321

Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (1µl/106 cells)  
Validation criteria: <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21428>  
ThermoFisher A-21428

Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor 488 (1µl/106 cells)  
Validation criteria: <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Recombinant-Polyclonal/A27034>  
ThermoFisher Cat: A27034

Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (1µl/106 cells)  
Validation criteria: <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11055>  
ThermoFisher Cat: A-11055

Sema3A Polyclonal Antibody, HRP Conjugated (2µl/106 cells)  
Validation criteria: <https://www.biossusa.com/products/bs-10468r-hrp>  
Bioss Antibodies Cat: bs-10468R-HRP

Mouse R-Spondin 2 Antibody (2µl/106 cells)  
Validation criteria: [https://www.rndsystems.com/products/mouse-r-spondin-2-antibody-773029\\_mab32661](https://www.rndsystems.com/products/mouse-r-spondin-2-antibody-773029_mab32661) R&D Systems Cat: MAB32661-100

Normal Goat IgG Control  
Validation criteria: [https://www.rndsystems.com/products/normal-goat-igg-control\\_ab-108-c](https://www.rndsystems.com/products/normal-goat-igg-control_ab-108-c)  
R&D Systems Cat: AB-108-C

Validation

All antibodies used are validated for detection of indicated proteins and specificity was confirmed by comparison to isotype control

staining. All antibodies used in this study were commercially available by vendors and have been validated by the manufacturers. Validation and specific validation criteria can be found at the following vendor websites above.

## Animals and other organisms

Policy information about [studies involving animals](#): [ARRIVE guidelines](#) recommended for reporting animal research

### Laboratory animals

In this study we used male and female mice that were 8-12 weeks of age of the following strains:

Mouse: C57BL/6 Jackson Laboratories JAX: 000664  
 RRID:IMSR\_JAX:000664  
 Mouse: B6.SJL Jackson Laboratories JAX: 002014  
 RRID:IMSR\_JAX:002014  
 Mouse: Sema3A FL/FL RIKEN Repository IMSR: RBRC01106  
 RRID:IMSR\_RBRC01106  
 Mouse: Nrp1 FL/FL Jackson Laboratories JAX: 005247  
 RRID:IMSR\_JAX:005247  
 Mouse: Cdh5-Cre Taconic  
 Laboratories Taconic: 13073  
 RRID:IMSR\_TAC:13073  
 Mouse: NRP1(VEGF-) Dr. Chenguang Gu N/A  
 Mouse: p53(-/-) Jackson  
 Laboratories JAX: 002101  
 RRID:IMSR\_JAX:002101

Animals were housed in a clean barrier facility with room temperature conditions (20-26 °C), relative humidity 30-70%, with 12-hour dark/light cycles.

### Wild animals

No wild animals were used in this study.

### Field-collected samples

No field-collected samples were used in this study.

### Ethics oversight

All animal procedures were performed in accordance with UCLA Animal Research Committee-approved protocol (ARC #2014-021-13P, principal investigator: JPC) and Cedars Sinai Medical Center Animal Use Committee-approved protocol (IACUC009617).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Flow Cytometry

### Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

#### Sample preparation

Detailed sample preparation and source of samples are listed in Methods.

#### Instrument

BD FACS Canto II and FACS Aria II

#### Software

BD FACSDIVA (BD Biosciences, NJ) for collection and FlowJo v9 (Treestar, Ashland, OR) for analysis.

#### Cell population abundance

10,000 cells were sorted and purity was assessed by expression of endothelial surface markers.

#### Gating strategy

Relevant gating strategies shown in corresponding flow plots and Supplementary Information. Positive gates were set to levels where isotype staining was <1%.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.